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Analytical Methods

Soybean seeds and its by-product okara as sources of dietary fibre. Measurement by AOAC and Englyst methods

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Abstract

The composition of soybean seeds and its by-product okara has been studied in this work. Dietary fibre was analysed by Englyst et al. method, by enzymatic–gravimetric methods of AOAC and by the quantification of the monomers obtained from the AOAC residues after acid hydrolysis (AOAC plus hydrolysis). Total dietary fibre by the enzymatic–gravimetric methods of AOAC in okara (55.48 g/100 g dry matter) is more than twice that of soybean seeds (24.37 g/100 g dry matter). The proportion IF/SF is 11 in okara and 6 in soybean seeds. Dietary fibre results from enzymatic–gravimetric AOAC methods are higher in okara and soybean seed samples than those from the Englyst method (okara: 41.14 g/100 g dry matter; soybean seeds: 15.05 g/100 g dry matter), and AOAC plus hydrolysis (okara: 44.91 g/100 g dry matter; soybean seeds: 16.38 g/100 g dry matter). In the case of the insoluble fibre from both samples, AOAC plus hydrolysis gives significantly ($p < 0.001$) higher values than the Englyst method, whilst for soluble fibre the opposite occurs ($p < 0.001$). The main monomers in soybean seeds and okara fibre are glucose, galactose, uronic acids, arabinose and xylose. The proportion of each monomer is similar in soybean seeds and okara, so the healthy properties of soybean seeds fibre are also claimed for okara.

Keywords: Dietary fibre; Non-starch polysaccharides; Soybean seeds; Okara

1. Introduction

It is well known that dietary fibre plays an important role in many physiological processes and in the prevention of diseases of different origin (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006). Increased dietary fibre intake can be achieved by changing dietary habits, increasing the consumption of high fibre foods and consuming fibre-fortified foods and fibre supplements. Fibre fortification of common foods has the advantage of requiring the least dietary changes for our fast food/convenience food consuming population (Lo, 1989).

Dietary fibre has all the characteristics required to be considered as an important ingredient in the formulation of functional foods (Heredia, Jiménez, Fernández-Bolaños, Guillén, & Rodríguez, 2002). The importance of food fibre has led to the development of a large potential market for fibre-rich products and ingredients and, in recent years, there is a trend to find new sources of dietary fibre that can be used as ingredients in the food industry (Chau & Huang, 2003). The most common fibre sources are bran from wheat, barley, corn, rice and oats; citrus fruits, grape, apple and sugar-beet fibre; soybean, peanut, pea and sunflower hull. Many of these sources of dietary fibre have been used in bread making and other cereal-based products (Anil, 2007).

On the other hand, while a few years ago the by-products generated during the processing of plant food constituted an economic and environmental problem, today they are considered a promising source of functional compounds (Carle et al., 2001). The components that remain

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after isolating the main constituent of the total by-products are abundant and represent an inexpensive material that has been undervalued until now, being only used as combustible or fertilizer (Grigelmo-Miguel & Martín-Belloso, 1999).

The most notable attributes of soybean seeds are their oil and protein contents, however, they are also a good source of dietary fibre and isoflavones. The production of soymilk and tofu results in a by-product called okara. In any country, discarding okara as waste is potentially an environmental problem because okara is highly susceptible to putrefaction. Okara also has a high moisture content (≈80%), making it difficult to handle and too expensive to dry by conventional means. In light of its content in dietary fibre, okara is a suitable candidate for nutritional enrichment of cereal-based products. Finding convenient ways to incorporate okara into food could eliminate a possible source of pollution and add economic value to this currently valueless product (Rinaldi, Ng, & Bennink, 2000).

Soybean seeds fibre is supposed to have good nutritional and functional properties. The neutral taste and the absence of colour of such fibres makes them suitable for incorporation into food products without any change in their quality, unlike those from wheat fibres (Rinaldi et al., 2000; Surel & Couplet, 2005).

In this study, we have focused on the determination of dietary fibre from soybean seeds and its by-product okara. Fibre has been analysed by the Englyst method (Englyst, Quigley, & Hudson, 1994) and enzymatic–gravimetric AOAC methods (1995). Furthermore the residues, insoluble and soluble, obtained by the AOAC methods have been subjected to the same hydrolysis and monomers quantification (GLC and spectrophotometry) that are utilized in the Englyst method. In addition, the proximate composition of both samples (soybean seeds and okara) has been considered.

2. Materials and methods

2.1. Materials

Soybean seed (*Glycine max* (L.) Merrill) and okara samples were supplied by Toofu-Ya, S.L. (Arganda del Rey, Madrid, Spain). Soybean seed samples were blended and homogenised by grinding to a fine powder to pass through 0.4 mm sieve and stored at 4 °C prior to analysis. Okara samples were freeze-dried (Freeze-dryer Telstar, mod. Cryodos) and prepared in the same way as soybean seed samples.

2.2. Proximate composition

Moisture was determined by oven-drying method at 105 ± 1 °C. Fat was measured in a Soxhlet system by extraction with diethyl ether solvent. Total nitrogen content was analysed by the Kjeldahl procedure. The conversion factor used to transform nitrogen into protein was 5.71. Ash content was determined by incineration of samples at 550 °C in a muffle furnace (James, 1995).

2.3. Dietary fibre

Enzymatic–gravimetric AOAC methods (AOAC., 1995): Isolation of dietary fibre (DF) was carried out with heat-stable alpha-amylase (termamyl) (pH 6, 100 °C, 30 min), protease (pH 7.5, 60 °C, 30 min) and finally amyloglucosidase (pH 4.5, 60 °C, 30 min). The obtained residues were filtered separating the liquid filtrate to soluble fibre (SF) analysis and the solid residue to insoluble fibre (IF) analysis. In both residues, SF and IF, the contents of protein and ash were calculated, and also dietary fibre content. *Englyst et al. method (1994)*: Isolation of DF was carried out with termamyl (pH 5.2, 100 °C, 10 min) followed by treatment with a mixture of pancreatin and pullulanase (pH 7.0, 50 °C, 30 min). Four residues of DF were obtained for each sample. Two were destined to total dietary fibre (TF) analysis, adding HCl 5 M and acidified ethanol (30 min, in ice bath), and the other two to IF analysis, adding phosphate buffer (30 min, 100 °C). The insoluble residues obtained were hydrolysed with H_2SO_4 12 M at 35 °C during 30 min followed by H_2SO_4 2 M at 100 °C during 1 h. The released neutral sugars were transformed into alditol acetates with acetic anhydride in the presence of 1-methylimidazol. Quantification was performed in a Perkin–Elmer Autosystem chromatograph equipped with a hydrogen flame ionization detector. The column used was a SP-2330 (30 m long, 0.25 mm i.d., and 0.25 μm film thickness) and nitrogen served as carrier gas (22 psi). Temperatures of injector and detector were 275 °C and oven temperature was 235 °C. Retention times and peak areas were registered in a PE Nelson computer mod. 1020 and β-D-allose (Fluka) was used as internal standard. Soluble fibre was calculated as the difference between TF and IF. *Uronic acids* content was determined in the acid hydrolysates according to the colorimetric method of 3,5-dimethylphenol as modified by Rodriguez, Redondo, and Villanueva (1992), with a Pharmacia mod. LKB Ultrospec Plus Spectrophotometer, using galacturonic acid (Merck) as standard. *AOAC plus hydrolysis*: SF and IF residues, from enzymatic–gravimetric methods (AOAC., 1995), were submitted to the same hydrolysis, derivatization to transform neutral sugars into alditol acetates derivates and GLC quantification, following the Englyst protocol (Englyst et al., 1994). Uronic acids were measured by the same colorimetric method (Rodriguez et al., 1992).

2.4. Statistical analysis

Results were calculated from the mean of six replicates and expressed in g/100 g dry matter. The significant differences between results from AOAC plus hydrolysis and the Englyst method were determined by analysis of variance

(ANOVA). A single linear regression analysis between both dietary fibre methods was performed.

3. Results and discussion

3.1. Proximate composition

The main components of soybean seeds are protein, dietary fibre and fat. Nearly 50% corresponds to proteins, 20% to fat and 25% to dietary fibre (Table 1). Karr-Lilienthal, Kadzere, Grieshop, and Fahey (2005) and Karr-Lilienthal et al. (2006) indicate that soybean seeds dry matter contain approximately 38–40% crude protein, 18–21% crude fat and 35% carbohydrates, approximately half of these carbohydrates are structural polysaccharides.

The proximate composition of the okara will depend on the procedure followed to obtain it. In this study, the okara has been obtained by the Japanese method. In this method, the rehydrated soybean seeds are cooked before grinding and filtering. The okara studied in this work is very rich in fibre (55.48 g/100 g), which would justify its use as a source of fibre, and also in protein, 28.52 g/100 g (Table 1). The protein in okara is of the same quality as that from other soy products. Wang and Cavins (1989) reported that the ratio of essential amino acids to total amino acids was similar to tofu and soy milk. Soybean seeds protein is one of the important vegetable protein resources due to its functional properties and high nutritional value (Liu, 1997). On the other hand, the okara is also rich in fat, 9.84 g/100 g, being specially important its high content of polyunsaturated fatty acids, linoleic and linolenic acids. Available data suggest that the diet of many people is poor in linolenic acid, so soybean seeds and okara, could be a good source to increase the linolenic acid intake. Other authors (O'Toole, 1999; Surel & Couplet, 2005; Van der Riet, Wight, Cilliers, & Datel, 1989) give similar values to those obtained in this work with respect to proteins (20–30%), ash (3–4%) and fibre (more than 50%). However the values given for fat vary in a very wide range (9–20%), with the values of the samples studied here being close to the lower limit.

Table 1
Proximate composition from soybean seeds and okara (% dry matter)

	Soybean seeds[a]	Okara	Sign[c]
Protein	46.06	28.52	***
Fat	20.03	9.84	***
Dietary fibre	24.37	55.48	***
Carbohydrates[b]	5.06	2.56	***
Ash	4.47	3.61	***

Mean values ($n = 6$).
[a] Soybean seeds moisture is 8.2%.
[b] Calculated from mean values by difference.
[c] Simple ANOVA: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, and ns: non significant.

Table 2
Gravimetric composition of residue material of soybean seeds and okara from AOAC Method (%)

	Soybean seeds	Okara	Sign[b]
Gravimetric residue IF			
Residue	27.13	56.54	***
Protein	5.78	4.98	*
Ash	0.49	0.79	ns
Insoluble fibre[a]	20.86	50.77	***
Gravimetric residue SF			
Residue	6.73	8.91	**
Protein	2.65	3.06	**
Ash	0.58	1.14	*
Soluble fibre[a]	3.5	4.71	**
Total dietary fibre	24.36	55.48	***
IF/SF	5.9	10.7	

Mean values ($n = 6$).
[a] Residue-protein-ash.
[b] Simple ANOVA: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, and ns: non significant.

3.2. Dietary fibre

Table 2 shows the composition of the insoluble and soluble residues obtained by the enzymatic–gravimetric methods of AOAC. (1995) for soybean seed and okara samples. The residual protein is present in the insoluble and soluble residues of the two types of samples, in a greater proportion in those resulting from soybean seeds for the insoluble residue but inversely for the soluble residue. With respect to the residual ash, its content is greater in the okara than in the soybean seeds but, contrary to the previous case, with lower values for the insoluble residue than in the soluble. These values of protein and ash are subtracted from the analytical residue weight according to the AOAC protocol. The efficiency of the protease in eliminating the protein present in the samples has been of 82% in soybean seeds and 72% in okara, calculated from the total protein content of each sample. In the case of the ash the analytical method eliminates 76% for the soybean seeds and 47% for the okara, calculated from the total contents.

The samples studied are a good source of dietary fibre since they have a total content very superior to the majority of the foods usually consumed (Table 2). The insoluble fibre (IF) of the okara is more than twice that of the soybean seeds. In both cases the content of soluble fibre is more similar, which results in a ratio IF/SF of 6 in soybean seeds and 11 in okara. The difference in the ratio could be attributed to the treatment of the soybean seeds to obtain the okara (temperature, time, quantity of water).

3.3. Monomers of dietary fibre

The dietary fibre was also analysed by the enzymatic–chromatographic method of Englyst et al. (1994) which determines non-starch polysaccharides (NSP). The Englyst NSP procedure uses gas–liquid chromatography measurement of neutral sugars as alditol acetate derivatives and

colorimetric measurement of uronic acids. The residues obtained by the AOAC methods (IF and SF) were subjected to the same acid hydrolysis and the monomers studied in the same way as the Englyst method (AOAC plus hydrolysis). The monomeric composition of the soybean seed and okara samples studied by both procedures are given in Tables 3–5. The content of each of the monomers of the insoluble, soluble and total fibre fractions is always higher for the okara than for the soybean seeds.

The insoluble dietary fibre of the samples studied (Table 3) is characterized by its high content in glucose, followed by galactose and arabinose, as well as important amounts of uronic acids and xylose. The remaining monomers, mannose, rhamnose and fucose, are present in smaller amounts. From this composition it can be deduced that the insoluble fibre comprises basically cellulose and hemicelluloses. In the latter polysaccharides, the arabinogalactan are strongly represented, as indicated by Hisamatsu, Fukumoto, Noda, Teranishi, and Yamada (1995). AOAC plus hydrolysis gives values slightly higher than the Englyst method, both for the soybean seeds and the okara. On studying the results obtained for the soybean seeds, the statistically significant differences are for rhamnose ($p < 0.05$), arabinose ($p < 0.001$), xylose ($p < 0.01$) and glucose ($p < 0.01$). For okara the differences between the results obtained by the two procedures are statistically significant in every case except for mannose ($p > 0.05$). The values of total monomers, for soybean seeds and okara, present significant differences depending on the procedure utilized ($p < 0.001$).

With respect to the soluble fibre (Table 4), galactose, uronic acids and arabinose are the most representative monomers. For the galactose, differences between the two samples are observed, with the okara having higher values than the soybean seeds. The uronic acids and the arabinose present similar values. Wang, Huang, Nakamura, Burchard, and Hallett (2005) indicate that the principal monomers in the soluble dietary fibre from soybean seeds are galactose 48%, arabinose 20% and galacturonic acid 20%. Pectic polysaccharides from soybean seeds and okara are comprised of regions of galacturonan and rhamnogalacturonan with rather large branched arabinogalactan side chains (Huisman, Schols, & Voragen, 1999; Yamaguchi,

Table 3
Monomeric composition of insoluble fibre in soybean seeds and okara (g/100 g dry matter)

	Soybean seeds			Okara		
	AOAC method plus hydrolysis[a]	Englyst method[b]	Sign[c]	AOAC method plus hydrolysis[a]	Englyst method[b]	Sign[c]
Rhamnose	0.25	0.21	*	0.55	0.47	*
Fucose	0.22	0.19	ns	0.41	0.37	*
Arabinose	1.72	1.43	***	5.74	5.17	**
Xylose	1.41	1.28	**	5.08	4.23	***
Mannose	0.5	0.45	ns	1.00	0.91	ns
Galactose	2.79	2.43	ns	9.04	8.31	*
Glucose	4.73	3.96	**	14.86	13.07	**
Uronic acids	1.61	1.37	ns	3.76	3.1	**
Total	13.23	11.32	***	40.44	35.63	***

Mean values ($n = 6$).
[a] Data determined by GC according to the Englyst procedure after enzymatic digestion described in gravimetric AOAC method.
[b] Data determined by GC according to the Englyst method.
[c] Simple ANOVA: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, and ns: non significant.

Table 4
Monomeric composition of soluble fibre in soybean seeds and okara (g/100 g dry matter)

	Soybean seeds			Okara		
	AOAC method plus hydrolysis[a]	Englyst method[b]	Sign[c]	AOAC method plus hydrolysis[a]	Englyst method[b]	Sign[c]
Rhamnose	0.12	0.15	**	0.30	0.37	*
Fucose	nd	nd		0.04	0.07	**
Arabinose	0.57	0.59	ns	0.61	0.73	*
Xylose	0.07	0.09	**	0.05	0.09	***
Mannose	0.21	0.25	**	0.26	0.28	ns
Galactose	1.08	1.27	**	1.79	2.11	*
Glucose	0.13	0.19	*	0.15	0.27	***
Uronic acids	0.98	1.19	**	1.27	1.6	**
Total	3.16	3.73	***	4.46	5.51	***

Mean values ($n = 6$).
[a] Data determined by GC according to the Englyst procedure after enzymatic digestion described in gravimetric AOAC method.
[b] Data determined by GC according to the Englyst method.
[c] Simple ANOVA: $^{***}p<0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, ns: non significant, and nd: not detected.

Table 5
Composition of total fibre in soybean seeds and okara (g/100 g dry matter)

	Soybean seeds			Okara		
	AOAC method plus hydrolysis[a]	Englyst method[b]	Sign[c]	AOAC method plus hydrolysis[a]	Englyst method[b]	Sign[c]
Rhamnose	0.37	0.35	ns	0.85	0.84	ns
Fucose	0.22	0.19	ns	0.45	0.44	ns
Arabinose	2.29	2.02	*	6.35	5.90	*
Xylose	1.48	1.38	**	5.14	4.32	***
Mannose	0.71	0.7	ns	1.26	1.19	ns
Galactose	3.87	3.7	ns	10.83	10.42	ns
Glucose	4.86	4.15	*	15.00	13.33	**
Uronic acids	2.58	2.56	ns	5.03	4.7	ns
Total monomers	16.38	15.05	***	44.91	41.14	***
Neutral NCP[d]	7.95	7.43	*	22.14	20.57	**
Acid NCP[e]	2.35	2.33	ns	4.58	4.28	ns
CP[f]	4.33	3.69	**	13.35	11.87	**
NSP[g]	14.63	13.46	***	40.07	36.71	***

Mean values ($n = 6$).
NCP = Non cellulosic polysaccharides, CP = Cellulosic polysaccharides, NSP = Non-starch polysaccharides.
[a] Data determined by GC according to the Englyst procedure after enzymatic digestion described in gravimetric AOAC method.
[b] Data determined by GC according to the Englyst method.
[c] Simple ANOVA: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, ns: non significant, and nd: not detected.
[d] Total neutral sugar values were expressed as neutral NCP by multiplying the amounts by 0.88 factor.
[e] Uronic acid values were expressed as acid NCP by multiplying the amounts by 0.91 factor.
[f] Glucose values were expressed as CP by multiplying the amounts by 0.89 factor.
[g] NSP content as sum of components.

Ota, & Hatanaka, 1996). Soybean seeds soluble polysaccharides are considered to have a globular structure with long neutral side chains of α-(1 → 3) and α-(1 → 5) arabinan and β-(1 → 4) galactan which are, respectively, composed of 20% and 49% of total sugars (Nakamura, Furuta, Kato, Maeda, & Nagamatsu, 2003).

Brillouet and Carre (1983) reported that total soybean seeds cell wall pectic substances contain arabinose and galactose in a molar ratio of 1:1.5. The arabinose:galactose ratio of soybean seeds is opposite to that of other legumes (1:0.7–0.9). Thus according to this ratio, for legumes there is a dominance of arabinose over galactose and for soybean seeds the opposite applies. In the samples studied in this work, the arabinose:galactose ratio is of 1:2 for the soybean seeds and 1:3 for the okara, which shows that the greater presence of galactose indicated by the previous authors is even more so in these samples.

The monomers of the soluble fibre (Table 4) have slightly greater values by the Englyst method; inversely to that which occurs in the analysis of the insoluble fraction (Table 3). On studying each of the monomers, it is observed that the differences between the results obtained by the two procedures are statistically significant except for the case of the arabinose for the soybean seeds and the mannose for the okara ($p > 0.05$). The total monomers values for both samples present significant differences between the methods ($p < 0.001$).

Since the insoluble fibre is the most representative of the total fibre (Table 5), the monomeric profile for both samples is similar, that is firstly glucose and galactose, then for soybean seeds uronic acids followed by arabinose but for okara in inverse order. Xylose follows with similar proportion in each sample. As for the insoluble fibre, the remaining monomers are much less representative. Table 5 gives the results obtained for the total fibre by both procedures with differences that are not statistically significant except in the cases of arabinose (soybean seeds and okara: $p < 0.05$), xylose (soybean seeds: $p < 0.01$ and okara: $p < 0.001$) and glucose (soybean seeds: $p < 0.05$ and okara: $p < 0.01$). For the insoluble fibre, AOAC plus hydrolysis gives higher values than the Englyst method, whilst for the soluble fibre the opposite occurs. For this reason, the quantitative differences between the two procedures decrease on studying the monomers of the total fibre.

The structural polysaccharides found in soybean seeds and okara are diverse. The specific structures of dietary fibre are not well understood. The monomer composition of the soybean seed and okara samples studied indicates the presence of cellulose, xylan, galactan, arabinan and galacturonan. In this matter, Karr-Lilienthal et al. (2005) indicate that soybean seeds dietary fibre is comprised of cellulose, pectin, and hemicelluloses, along with mannans, galactans, and xyloglucans.

Table 5 shows the polysaccharides that make up the dietary fibre. It indicates that the most representative for both samples are the neutral non-cellulosic polysaccharides (neutral NCP). In the case of the soybean seeds, the values for acid non-cellulosic polysaccharides (acid NCP) and cellulosic polysaccharides (CP) are closer, whilst for the okara the CP clearly predominate over the acid NCP. In the same manner as when comparing the methods for quantifying the monomers, AOAC plus hydrolysis gives higher values

than the Englyst method, with statistically significant differences except for the acid NCP ($p < 0.05$). It is worth highlighting that the differences observed for the NSP by each method present the same significance for soybean seeds and okara ($p < 0.001$).

Comparing the AOAC plus hydrolysis procedure and the Englyst method for the analysis of the total fibre in the samples of soybean seeds and okara has yielded the corresponding regression lines ($y = bx + a$) and R^2 values (Table 6). The intercept values on the ordinate are close to zero for all the different monomers. The values for the slope are close to 1 which indicates that both methods quantify in a similar manner, although the results obtained by AOAC plus hydrolysis are always slightly higher. The R^2 values are high for all the monomers, especially for the arabinose, galactose and glucose, and lower for the mannose. For the total fibre, the fit of the points on the line is $R^2 = 0.991$.

The comparison of the results obtained for the total fibre by AOAC. (1995) (Table 2) and Englyst (Englyst et al., 1994) (Table 5) methods, indicates that the enzymatic–gravimetric method yields much higher values (soybean seeds: 24.36 g/100 g, okara: 55.48 g/100 g) than the enzymatic–chromatographic method (soybean seeds: 15.05 g/100 g, okara: 41.14 g/100 g). Some authors (Gray, 2006; Kontraszti, Hudson, & Englyst, 1999) confirm that the enzymatic–gravimetric methods approved by the AOAC generate significantly higher values for dietary fibre than the Englyst method, particularly for foods rich in starch. The stated objectives of the AOAC and the Englyst methods are different and as they are designed to include different fraction of foods, different values are therefore to be expected. The AOAC method is based on the concept of resistance to digestion. It uses enzymatic digestion to eliminate non-fibre components and quantification of the residues by weighing, whereas the Englyst procedure is designed to measure non-starch polysaccharides (NSP) and to identify the different monomers that make up the insoluble and soluble fibre.

Having confirmed the large differences between the enzymatic–gravimetric methods of the AOAC. (1995) and the Englyst method (Englyst et al., 1994), the residues corresponding to the IF and SF were hydrolysed to determine the monomeric composition of the polysaccharides present in them. The total fibre, sum of the IF and SF, for the soybean seeds is 24.36 g/100 g (AOAC method) (Table 2) compared with total monomers of 16.38 g/100 g (AOAC plus hydrolysis) (Table 5) and for the okara is 55.48 g/100 g (AOAC method) (Table 2) compared with 44.91 g/100 g (AOAC plus hydrolysis) (Table 5). These differences indicate that an important part of the residue is not polysaccharides. The fact that lignin is not included in the non-starch polysaccharides analysis contributes further to the lower values for this method. Englyst, Quigley, Englyst, Bravo, and Hudson (1996) used the AOAC method and after correction of the residue weight for ash and crude protein, and separate measurement of Klason lignin, starch and NSP, from 5% to 42% of the residue weight remained unaccounted for.

The analytical method utilized to quantify the dietary fibre has an influence upon the results obtained and therefore it must be taken into account when recommending the intake of fibre. The best definition of dietary fibre is still to be established and this fact, jointly with other factors that can have an influence like the analytical method used to measure fibre, the origin of the particular fibre and the specific individual characteristics, should be taken into account when establishing the recommended intakes in order to guarantee the potential health benefits of the same. The recommended intake of fruits and vegetables and consumption of wholegrain foods is likely to provide >20 g per day of NSP (Englyst method) and >25 g per day of total dietary fibre (AOAC gravimetric methods) (World Health Organization., 2003).

This work has shown that very different values for dietary fibre are obtained by the enzymatic–gravimetric AOAC methods and by the enzymatic–chromatographic methods studied and that this leads to quite different fibre values for identical samples as well as to the calculation of very different fibre intakes. On the other hand, the results obtained suggested that okara can be used as a functional ingredient since its high content of dietary fibre, along with the presence of fat and proteins of high quality, as well as the absence of colour and taste, makes it suitable for incorporation into food products.

Table 6
Single linear regression analysis between AOAC plus hydrolysis and Englyst methods

	$y = bx + a$	R^2
Rhamnose	$y = 1.0018x - 0.0127$	0.953
Fucose	$y = 1.0013x - 0.0238$	0.914
Arabinose	$y = 0.9482x - 0.1302$	0.995
Xylose	$y = 0.7977x + 0.2152$	0.941
Mannose	$y = 0.7869x + 0.1715$	0.710
Galactose	$y = 0.9620x - 0.0144$	0.968
Glucose	$y = 0.8995x - 0.1938$	0.967
Uronic acid	$y = 0.8586x - 0.3673$	0.948
Dietary fibre	$y = 0.9113x - 0.1802$	0.991

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